

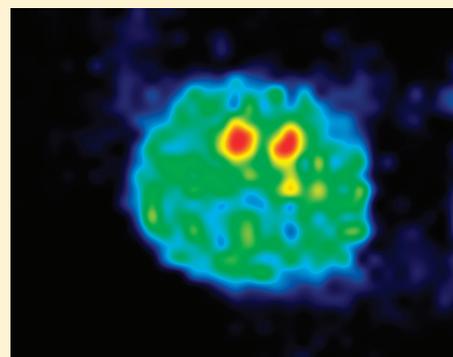
Adenosine A_{2A} Receptor Antagonists and Parkinson's Disease

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ABSTRACT: This Review summarizes and updates the work on adenosine A_{2A} receptor antagonists for Parkinson's disease from 2006 to the present. There have been numerous publications, patent applications, and press releases within this time frame that highlight new medicinal chemistry approaches to this attractive and promising target to treat Parkinson's disease. The Review is broken down by scaffold type and will discuss the efforts to optimize particular scaffolds for activity, pharmacokinetics, and other drug discovery parameters. The majority of approaches focus on preparing selective A_{2A} antagonists, but a few approaches to dual A_{2A}/A₁ antagonists will also be highlighted. The in vivo profiles of compounds will be highlighted and discussed to compare activities across different chemical series. A clinical report and update will be given on compounds that have entered clinical trials.

KEYWORDS: Adenosine, A_{2A} receptor antagonist, A₁ receptor antagonist, Parkinson's disease, catalepsy, 6-OHDA, MPTP



Parkinson's disease (PD) is a chronic, progressive neurological disease that affects ~1% of the population over the age of 65.¹ It is characterized by progressive impairment in motor function that is often accompanied by disturbances in mood and cognitive function. The majority of motor impairments of PD are caused by a gradual loss of dopamine (DA) producing neurons in the ventral midbrain and concomitant loss of DA input to forebrain (striatal) motor structures.^{2,3} The loss of DA input to the neostriatum leads to dysregulation of striatal function and the classic motor symptoms of PD, such as resting tremor, muscular rigidity, and bradykinesia.

The majority of treatments aim to restore dopamine signaling and thereby reduce the severity of the motor symptoms. Dopamine replacement therapy using L-DOPA, the precursor to dopamine, remains the gold-standard treatment for PD. Other approaches include inhibition of DA turnover using monoamine oxidase type B (MAO-B) inhibitors,⁴ catechol O-methyl-transferase (COMT) inhibitors,⁵ and inhibition of dopamine reuptake⁶ or direct agonists⁷ of postsynaptic dopamine receptors. Although the dopamine targeted therapies work well to address the PD related motor disturbances, they all produce undesirable side effects (dyskinesia, hallucinations, on-off effects) that become more severe and problematic with continued treatment. Also, the aforementioned therapies typically show reduced efficacy as motor functions deteriorate and the disease progresses. Moreover, these treatments do not alter disease progression and do not address the mood, postural instability, or cognitive disturbances that frequently accompany PD.

The dopamine replacement agents have significant limitations which influenced researchers to find nondopamine based treatments for PD. One nondopaminergic approach that has received considerable attention is modulation of adenosine receptors which is the topic highlighted in this Review.⁸ Adenosine is a

neuromodulator that coordinates responses to dopamine and other neurotransmitters in areas of the brain that are responsible for motor function, mood, and learning and memory.⁹ Adenosine comprises four distinct receptor subtypes designated A₁, A_{2A}, A_{2B}, and A₃ belonging to the G protein-coupled receptor superfamily.¹⁰ Adenosine A₁ and A₃ receptors are coupled to inhibitory G proteins, while A_{2A} and A_{2B} receptors are coupled to stimulatory G proteins. Autoradiography studies in rodents showed that the greatest densities of A_{2A} receptors are found in the striatum¹¹ which closely matches the distribution in humans based on PET imaging.¹²

As described above, loss of dopamine input into the neostriatum is a hallmark of PD and causes many of the cardinal motor symptoms of this disorder. In the striatum adenosine A_{2A} receptors colocalize and physically associate with dopamine D₂ receptors.^{2,3} A_{2A} and D₂ receptors have opposing effects on adenylyl cyclase and cAMP production in cells, such that activation of A_{2A} receptors inhibits dopamine D₂ receptor signaling. Conversely, A_{2A} receptor antagonists enhance D₂ dependent signaling as shown by induction of immediate early gene *c-fos* expression in the striatopallidal pathway¹³ and facilitate other D₂ mediated responses. Of importance to PD, pharmacological blockade of A_{2A} receptors has shown dramatic beneficial effects in preclinical animal models of PD, showing potentiation of dopamine-mediated responses in dopamine depleted (6-OHDA-treated)¹⁴ animals and dramatic relief of parkinsonian symptoms in MPTP-treated¹⁵ nonhuman primates.¹⁶ A_{2A} antagonists facilitate dopamine receptor signaling and thereby normalize motor function in animal models of dopamine dysregulation. As a result of these

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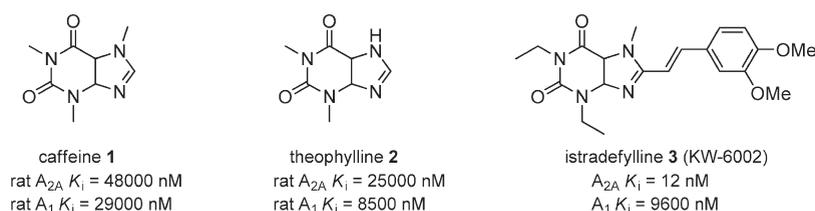


Figure 1. Xanthine based A_{2A} antagonists.

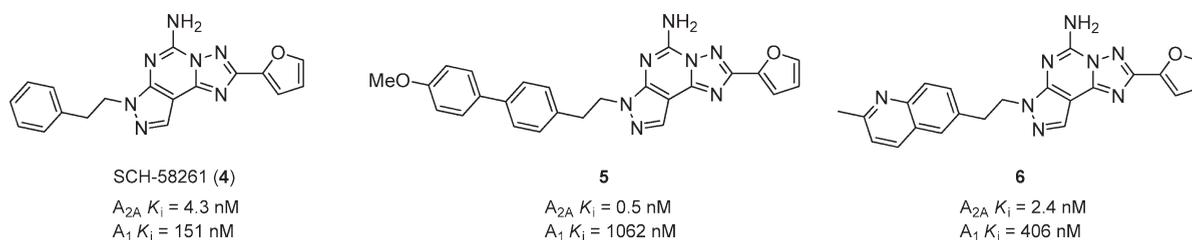


Figure 2. Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines.

findings, the adenosine A_{2A} receptor has become a sought after target for treating PD. Blockade of A_{2A} signaling by selective A_{2A} receptor antagonists¹⁷ was shown to be beneficial for not only enhancing the therapeutic effects of L-DOPA but also reducing dyskinesia from long-term L-DOPA treatment.¹⁸

The adenosine A_1 receptor is also expressed in the striatum; based on anatomical and in vivo microdialysis studies, A_1 receptors appear to be localized presynaptically of dopamine axon terminals where they inhibit dopamine release.¹⁹ A_1 receptor antagonists facilitate dopamine release in the striatum, and like A_{2A} receptors potentiate dopamine mediated responses. Antagonism of both the A_{2A} and A_1 would be synergistic: inhibition of the A_1 receptor will facilitate dopamine release, while inhibition of the A_{2A} receptor will enhance postsynaptic responses to dopamine. Interestingly, the A_1 receptor is also concentrated in neocortical and limbic system structures that are important for cognitive function. Pharmacological inhibition of A_1 receptors enhances neurotransmitter release in the hippocampus²⁰ and enhances performance in animal models of learning and memory.²¹ Antagonism of A_1 receptors could present a potential liability, as it is known that some A_1 antagonists have cardiovascular²² and diuretic²³ liabilities.

The major unmet medical needs of PD are as follows: improved symptomatic treatment without inducing adverse effects (primarily dyskinesia) associated with long-term L-DOPA or dopamine agonist therapy; opportunity to slow disease progression by protecting midbrain dopamine and other neurons from degeneration; treatment of disease comorbidities, including cognitive dysfunction, anxiety, and depression. Based on published preclinical and human clinical data, the majority of approaches focus on selective antagonism of A_{2A} receptors while a few approaches suggest that a dual A_{2A}/A_1 receptor antagonist may be better suited to address many of these unmet needs. There have been a number of excellent reviews on A_{2A} receptor antagonists reported in the literature,²⁴ but the following Review will highlight the work done on this target in the past five years. The K_i values represented in the figures refer to binding affinities unless noted otherwise. Updates on key compounds that have entered the clinic will also be highlighted.

■ XANTHINE BASED A_{2A} ANTAGONISTS

Adenosine A_{2A} receptor antagonists have been traditionally categorized as xanthine based or nonxanthine based derivatives. Caffeine (**1**) and theophylline (**2**) are two of the oldest known adenosine antagonists, and both have weak affinity for A_{2A} and A_1 (Figure 1).²⁵ Numerous efforts to identify xanthine based derivatives have been explored but will not be discussed in this Review.²⁶ However, istradefylline (**3**), KW-6002, is a xanthine based, selective A_{2A} antagonist that showed efficacy in numerous preclinical models of PD²⁷ and was investigated in several clinical trials for PD.²⁸ Istradefylline completed phase III clinical trials in 2009, but it was not approved by the FDA due to lack of efficacy.²⁹ There are two active phase III studies using istradefylline (clinicaltrials.gov identifier: NCT00955526 and NCT00957203). More recent efforts have focused on nonxanthine based scaffolds to identify viable selective A_{2A} and dual A_{2A}/A_1 receptor antagonists.

■ TRICYCLIC A_{2A} ANTAGONISTS

Schering-Plough identified a new series of nonxanthine based derivatives that led to SCH-58261 (**4**) as a potent A_{2A} antagonist with moderate 35-fold selectivity over the A_1 receptor (Figure 2).³⁰ SCH-58261 showed efficacy in rodent models of PD after intraperitoneal (i.p.) administration, but it suffers from poor solubility and is not active when dosed orally. There have been numerous efforts to increase the selectivity and pharmacokinetic (PK) properties of this particular scaffold. A series of biaryl substituted pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines was also prepared.³¹ The methoxy biaryl compound **5** had very potent affinity for A_{2A} and is significantly more selective (2124-fold) selective against the A_1 receptor compared to **4**. This compound showed 30 and 5% inhibition of rat catalepsy at 1 and 4 h, respectively, at 1 mg/kg, and exhibited very poor plasma exposure. Efforts to replace the methoxyaryl substituent with heterocycles such as pyridines and pyrazines resulted in compounds that retained potency and selectivity, but generally suffered from poor solubility and poor exposures in rat after oral dosing. Contracting the biaryl substituent into a quinoline,

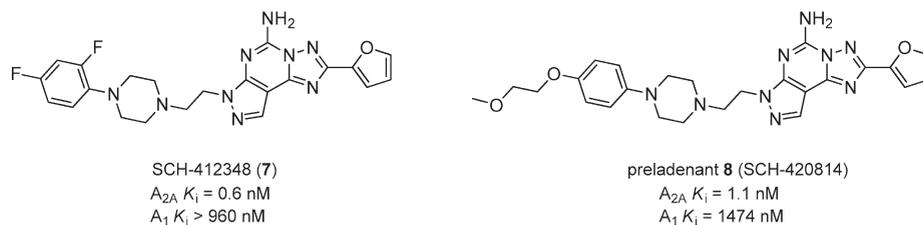


Figure 3. Aryl piperazine substituted pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines.

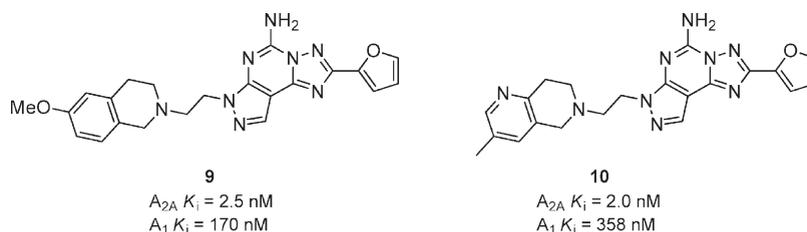


Figure 4. Fused heterocyclic substituted pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines.

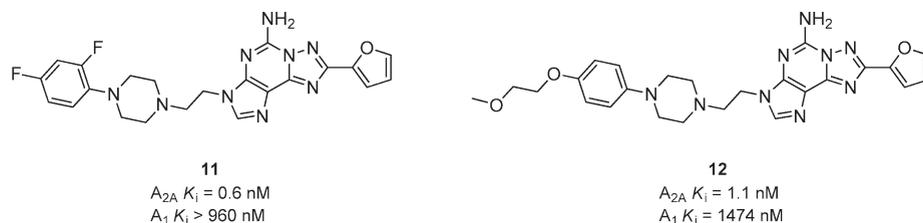


Figure 5. Aryl piperazine substituted 3*H*-[1,2,4]-triazolo[5,1-*i*]purin-5-amines.

represented in **6**, gave potent compounds with good selectivity versus A_1 . More importantly, **6** exhibited a superior PK profile in rats, compared to **4** and **5**, and reversed catalepsy in rats by 86% (1 h) and 38% (4 h) after 3 mg/kg, p.o.

The most successful strategy replaced the phenethyl side chain (compounds **4**–**6**) with an aryl-piperazine side chain represented in **7** (Figure 3).³² A very promising compound, SCH-412348 (**7**), showed very potent A_{2A} affinity and impressive selectivity (>1600-fold) versus A_1 . Although SCH-412348 showed excellent ability to reverse haloperidol-induced catalepsy (75 and 80% inhibition at 1 and 4 h, respectively) in rats after a 1 mg/kg dose, po, the compound was not progressed further due to poor aqueous solubility. Further optimization led to the methoxyethoxy analogue **8** (SCH-420814, preladenant), which also had excellent binding affinity for A_{2A} with good selectivity (1340-fold) over A_1 and improved solubility. Preladenant (1 mg/kg) reversed haloperidol-induced catalepsy (77 and 70% inhibition at 1 and 4 h, respectively) in rats and was further characterized in multiple preclinical animal models of PD.³³ Preladenant showed significant activity when dosed orally in the rat hypolocomotion model (minimum effective dose 0.1 mg/kg, p.o.), 6-OHDA-lesioned rat model (minimum effective dose 0.03 mg/kg, p.o.), and MPTP-treated monkey model.²⁴ Preladenant has completed phase II clinical trials for PD, and Merck (Schering-Plough) is currently recruiting for several phase III trials (clinicaltrials.gov identifier: NCT01155479, NCT01227265, NCT01155466, NCT01294800, NCT01215227, NCT01323855).³⁴

More recently a variety of fused heterocyclic derivatives of the tricyclic scaffold were prepared that had comparable A_{2A} binding affinities with respect to the aryl piperazines **7** and **8** but were generally much less selective against the A_1 receptor.³⁵ The tetrahydroisoquinoline derivative **9** had excellent binding affinity and was 68-fold selective versus A_1 (Figure 4). The compound had moderate plasma exposure in rats and reversed catalepsy in rat by 50 and 58% at 1 and 4 h, respectively, after a 3 mg/kg dose, p.o. A related azaisoquinoline **10** had a > 5-fold higher exposure in rat compared to **9** and reversed catalepsy in rat (3 mg/kg) by 85 and 30% at 1 and 4 h, respectively. A variety of substituted isoindolines and benzazepines were also prepared.

Replacing the pyrazole ring, present in the previously described scaffolds, with an imidazole ring gave a series of 3*H*-[1,2,4]-triazolo[5,1-*i*]purin-5-amines (Figure 5).³⁶ Compounds **11** and **12** contain the optimal aryl piperazine substituents present in the pyrazolopyrimidines **7** and **8**. Both **11** and **12** have good affinities for A_{2A} while maintaining good selectivity versus A_1 (88- and 669-fold, respectively), albeit considerably lower than the selectivities obtained from **7** and **8**. Compound **12** (1 mg/kg, p.o.) showed 55 and 50% inhibition of catalepsy in rat at 1 and 4 h, respectively, but in general the compounds from this series were inferior to the corresponding compounds from the pyrazolopyrimidine scaffold. Efforts to replace the furan substituent with substituted aryls resulted in compounds having good A_{2A} potency but were much less selective versus A_1 receptors.

A series of methylene amine substituted arylindenopyrimidines was reported as dual A_{2A}/A_1 receptor antagonists (Figure 6).³⁷

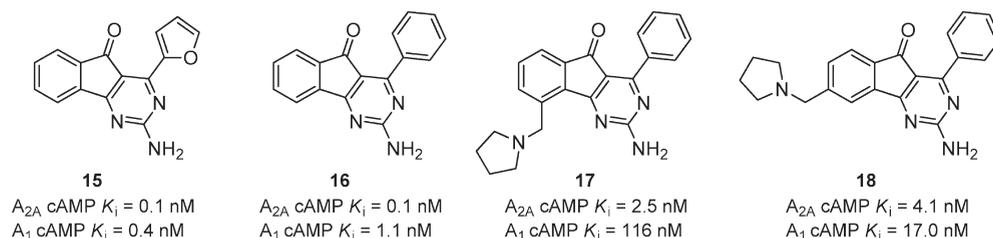


Figure 6. Arylindenopyrimidines.

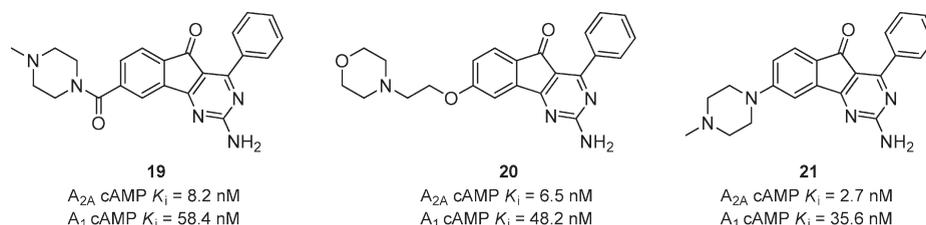
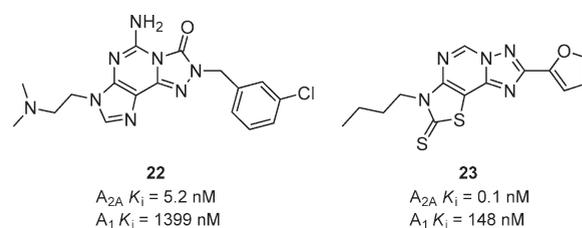


Figure 7. Substituted arylindenopyrimidines.

Compound **15** was the original lead compound that was potent in both A_{2A} and A_1 functional assays and had an ED_{50} of 5.0 mg/kg, p.o. in the haloperidol-induced catalepsy model in mice. Compound **15** suffered from poor solubility, and it was found to be Ames positive. A variety of heterocyclic furan replacements were prepared that generally maintained good in vitro potency but lost in vivo activity. Replacing the furan with phenyl gave **16** that had good in vitro potency and comparable in vivo activity (ED_{50} = 8.0 mg/kg, p.o.) reversing catalepsy in mice. Compound **16** was negative in the Ames screen, but it still suffered from poor solubility. A variety of amines were incorporated at the 9-position of the scaffold to increase solubility and resulted in the synthesis of **17** that had good in vitro potency and had an ED_{50} of 3.8 mg/kg, p.o. in the mouse catalepsy model. Moving the pyrrolidine to the 8-position gave compound **18** that was equipotent at A_{2A} , more potent at A_1 , and had significantly increased potency in vivo (ED_{50} = 0.2 mg/kg, p.o.) in the mouse catalepsy model. Further characterization of **18** showed that it was active in rat catalepsy (ED_{50} = 0.5 mg/kg, p.o.), and had minimum effective doses of 1.0, 1.0, and 10 mg/kg for reserpine-induced akinesia model in mice, 6-OHDA lesion model in rats, and reversing motor disability in MPTP-treated marmosets, respectively.³⁸

Further evaluation revealed that metabolism of **18** resulted in the formation of reactive metabolites that were attributed to some adverse events seen in the 28-day GLP toxicology studies in nonhuman primates.³⁹ The development of **18** was discontinued based on these findings, and the focus was to identify a suitable back up compound devoid of the metabolic liabilities. Oxidative metabolism was occurring on the pyrrolidine ring and at the benzylic methylene, so a number of molecules were synthesized to address this issue (Figure 7).³⁹ Compound **19** is a representative amide that had good functional activity at both A_{2A} and A_1 receptors and reversed haloperidol-induced catalepsy in mice (ED_{50} = 0.4 mg/kg, p.o.). The ether linked compound **20** was very potent in vivo with an ED_{50} < 0.1 mg/kg, p.o. in the mouse catalepsy model. A tissue distribution study in rats (10 mg/kg, p.o.) showed that **20** had a brain C_{max} of 4.1 μ M and a brain to plasma ratio of 3:1. The amino linked compound **21** was potent

Figure 8. Pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidon-3-one and thiazolotriazolopyrimidines.

in vitro and reversed catalepsy in mice with an ED_{50} < 1.0 mg/kg, p.o. All of the compounds were devoid of the metabolic liabilities associated with compound **18**.

A variety of ethylamino derivatives were prepared in a pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidon-3-one series (Figure 8).⁴⁰ The ethylenedimethylamino analogue **22** has good binding affinity for A_{2A} and is 269-fold selective versus A_1 receptors. In general, analogues had good binding affinities but were not able to reverse haloperidol-induced catalepsy in rat at 10 mg/kg, p.o., and showed very low exposures in rat brain. A series of thiazolotriazolopyrimidines was developed with multiple compounds having sub-nanomolar binding affinities.⁴¹ Compound **23** had potent A_{2A} binding affinity and exhibited good selectivity against A_1 receptors. Compound **23** also showed very potent functional (cAMP) activity (K_i = 0.1 nM) and was able to reverse both haloperidol-induced catalepsy and akinesia in mice after a 10 mg/kg dose, p.o.

■ BICYCLIC A_{2A} ANTAGONISTS

Substituted 1,2,4-triazolo[1,5-*c*]pyrimidines are a bicyclic system related to preladenant without the fused pyrazole ring (Figure 9).⁴² A variety of linkers were explored including O, S, NH, NMe, and NEt corresponding to compounds **24**–**28** while keeping the optimized aryl piperazine, present in preladenant, fixed.³⁹ In general, the binding affinities for A_{2A} were good for all of the linkers with good selectivity versus A_1 , but the O and S

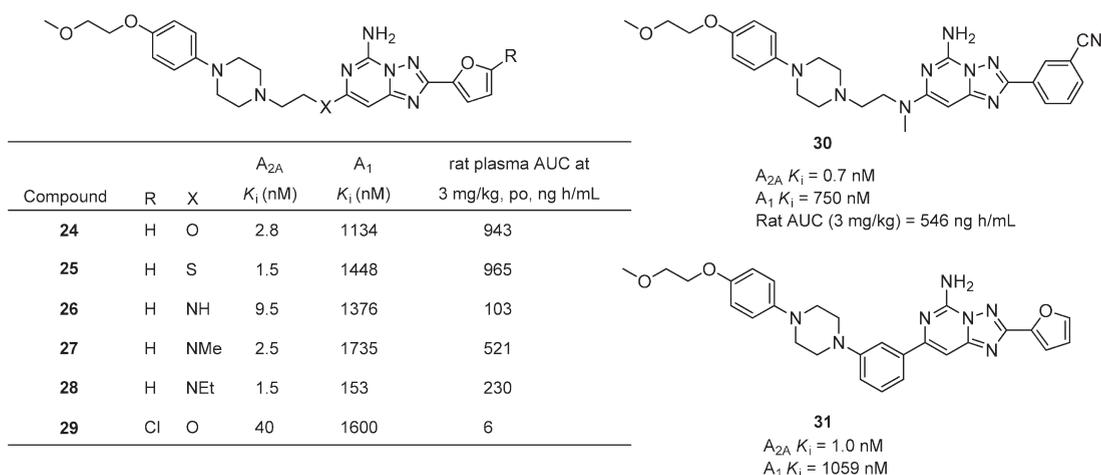


Figure 9. Substituted 1,2,4-triazolo[1,5-*c*]pyrimidines.

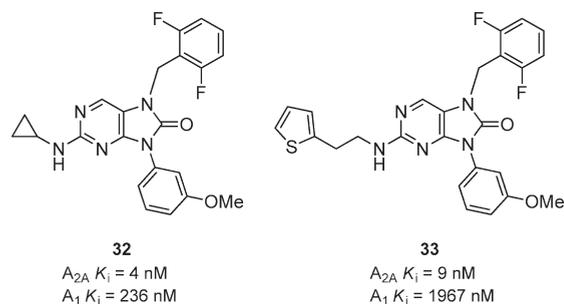


Figure 10. Substituted purinones.

linkages gave the best plasma exposures in rats compared to any of the nitrogen linked analogues. Interestingly, a chlorine substituent (**29**) on the furan significantly reduced A_{2A} activity and dramatically reduced plasma exposure compared to the *des*-chloro analogue **24**. Replacement of the furan in **24** with 3-cyanophenyl afforded compound **30** that had excellent binding affinity for A_{2A}, good selectivity versus A₁, and nearly identical exposure in rats. The compounds were also evaluated in haloperidol-induced catalepsy model in rats. Compound **25** gave the best results with 65% inhibition of catalepsy at both 1 and 4 h after 3 mg/kg, p.o. A related series of substituted 7-phenyl-[1,2,4]-triazolo[1,5-*c*]pyrimidine-5-amines was also reported.⁴³ Compound **31** was identified as having potent affinity for A_{2A} and good selectivity versus A₁. Further evaluation showed that **31** significantly reversed haloperidol-induced catalepsy at 3 mg/kg, p.o.

A series of trisubstituted purinones was discovered and elucidated from a high throughput screen (Figure 10).⁴⁴ An initial structure–activity relationship (SAR) was developed by substituting N-7 and N-9 with various aryl and benzyl substituents while keeping the cyclopropyl amine substitution at the 2-position constant. Compound **32** was the most potent compound for A_{2A} and was 59-fold selective versus A₁ receptors. Replacement of the cyclopropyl amine with 2-(thiophen-2-yl)ethanamine afforded compound **33** that had comparable activity for A_{2A} but was significantly more selective (218-fold) versus A₁ compared to compound **32**. The thiophene analogue also had good functional activity (cAMP) in rat (K_i = 18 nM), but no in vivo results were reported for any compounds within this series.

A series of thieno[3,2-*d*]pyrimidine-4-methanone derivatives was prepared as adenosine A_{2A} receptor antagonists with modest selectivity over A₁ (Figure 11).⁴⁵ The most potent compound **34** was potent at both A_{2A} and A₁, and was found to be active in haloperidol-induced hypolocomotion model in mice when dose at 30 mg/kg, i.p. Removing the ketone linker and directly attaching the heterocycle to the pyrimidine core afforded a series of 4-arylthieno[3,2-*d*]pyrimidines having good affinity for A_{2A} receptors.⁴⁶ Compound **35** was moderately potent for A_{2A} (K_i = 69 nM), but it did not reverse haloperidol-induced hypolocomotion in mice following i.p. administration of 30 mg/kg. Interestingly, replacing the 2-thienyl substituent with 2-thiazolyl resulted in a significant increase in A_{2A} binding affinity (K_i = 1.6 nM) and selectivity over A₁. Compound **36** was also able to reverse hypolocomotion in mice at 30 mg/kg, i.p. A similar aminopyrimidine **37** was identified that had good receptor binding for A_{2A} but was moderately selective versus A₁. Efforts to identify other bicyclic templates identified pyrazolo[3,4-*d*]pyrimidines **38** and **39** and 6-arylpurines **40** and **41**.⁴⁷ The pyrazolo analogues **38** and **39** both show good affinity for A_{2A} and were both able to reverse haloperidol-induced hypolocomotion in mice at 30 mg/kg, i.p. None of the analogues within this series, however, were active when given orally. Interestingly, the 6-aryl-purine **40** had much weaker affinity for A_{2A} but was effective in reversing hypolocomotion in mice when dosed at 10 mg/kg, i.p., and 1 mg/kg, p.o. No plasma or brain exposures were reported to explain this unexpected result. The benzyl analogue **41** was also active in vivo at 30 mg/kg, i.p, but not active when dosed orally.

Related benzyl substituted triazolo[4,5-*d*]pyrimidines like **42** were synthesized and evaluated (Figure 12).⁴⁸ Compound **42** had potent A_{2A} activity and exhibited moderate selectivity versus A₁. A variety of substituted benzyl analogues were prepared that had good A_{2A} activity but were not able reverse haloperidol-induced hypolocomotion when dosed orally. Inactivity was attributed to low solubility and poor PK profiles and led to the synthesis of heterocyclic compounds such as the 2-pyridyl analogue **43**. Despite good in vitro activity and increased solubility, **43** was not active in vivo as 30 mg/kg, p.o. Further optimization of this scaffold led to the discovery of vipadenant (**44**) (V2006/BIIB-014) which was evaluated in phase II clinical trials.⁴⁹ Vipadenant showed excellent binding affinity for A_{2A} and had

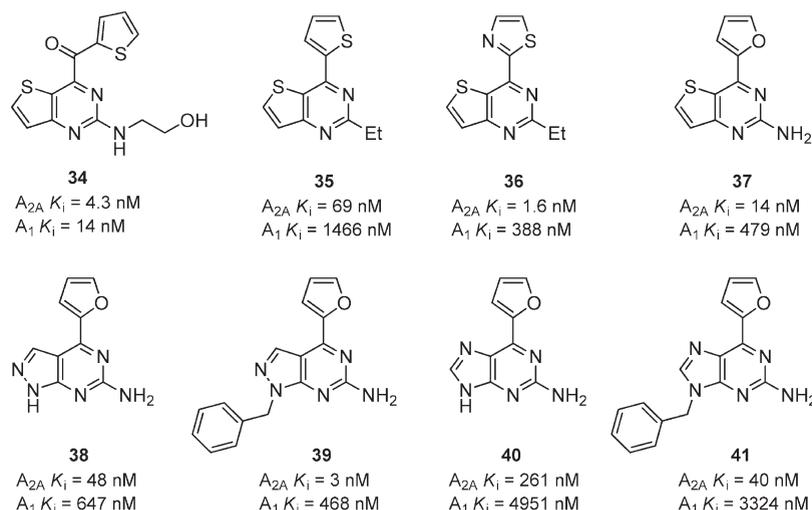


Figure 11. Thieno[3,2-*d*]pyrimidines, pyrazolo[3,4-*d*]pyrimidines, and 6-arylpyrimidines.

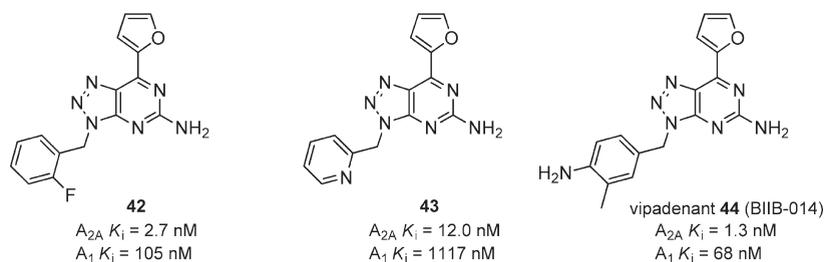


Figure 12. Benzyl substituted triazolo[4,5-*d*]pyrimidines.

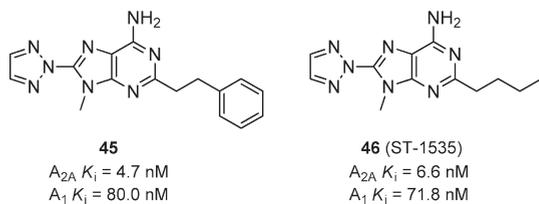


Figure 13. Triazolo-9*H*-purines.

modest selectivity against A_1 and A_{2B} ($K_i = 63 \text{ nM}$), while it showed good selectivity against A_3 ($K_i = 1005 \text{ nM}$). When tested *in vivo* in the mouse and rat haloperidol-induced hypolocomotion models, **44** had a minimum effective dose of 0.1 and 1 mg/kg, respectively. Vipadenant was able to increase contralateral rotations in 6-OHDA lesioned rats when dosed orally in combination with apomorphine at 3 and 10 mg/kg.^{24a} The compound also showed reversal of motor disability in MPTP-treated marmosets without dyskinesias at a minimum effective dose of <5 mg/kg, *p.o.*^{24a} The excellent *in vivo* data and a favorable safety profile helped progress this compound through phase II clinical trials. Despite positive phase II clinical results, in July 2010 Vernalis and Biogen have discontinued the development of vipadenant due to some preclinical toxicology findings that showed adverse side effects.⁵⁰

A series of triazolo-9*H*-purines was reported as potent A_{2A} antagonists having modest selectivity versus A_1 (Figure 13).⁵¹ Compounds **45** and **46** (ST-1535) are representative examples of the series developed by Sigma Tau. ST-1535 has been

characterized extensively in various animal models of PD. ST-1535 has minimum effective doses of 5.0 and 1.25 mg/kg in hypolocomotion and haloperidol-induced catalepsy models in mice, respectively.⁵² Further studies show that ST-1535 potentiated L-DOPA activity in 6-OHDA lesioned rats.⁵³ In the MPTP-treated marmosets, ST-1535 dosed alone at 40 mg/kg significantly increased locomotor activity but did not improve motor disability.⁵⁴ However, when dosed at 20 mg/kg in combination with L-DOPA (2.5 mg/kg), a significant reversal of motor disability was seen compared to 20 mg/kg alone of ST-1535. The significance was not reported with respect to L-DOPA (2.5 mg/kg) alone. ST-1535 is currently under active development in phase I clinical trials by Sigma Tau.

A series of aminomethyl substituted thieno[2,3-*d*]pyrimidines has been reported as A_{2A} antagonists having moderate selectivity versus A_1 (Figure 14).⁵⁵ The *in vitro* activities for A_{2A} and A_1 are reported as functional activity, not binding affinity. The unsubstituted furan proved to be the optimal substituent for A_{2A} potency and selectivity versus A_1 . The basicity of amine substituent was the key to obtain *in vivo* activity in the haloperidol-induced catalepsy model in mice. Very basic amines such as pyrrolidines and piperidines had good *in vitro* potency but were not able to reverse catalepsy *in vivo* at 10 mg/kg, *p.o.* The morpholine compound **47** was much less basic, based on calculated pK_a values, than the corresponding pyrrolidines and piperidines and had a reported oral ED_{50} of 1.3 mg/kg in the mouse catalepsy model. A variety of less basic amines were explored to determine the scope and to identify if the basicity was indeed a real trend. Compounds **48** and **49** were even more

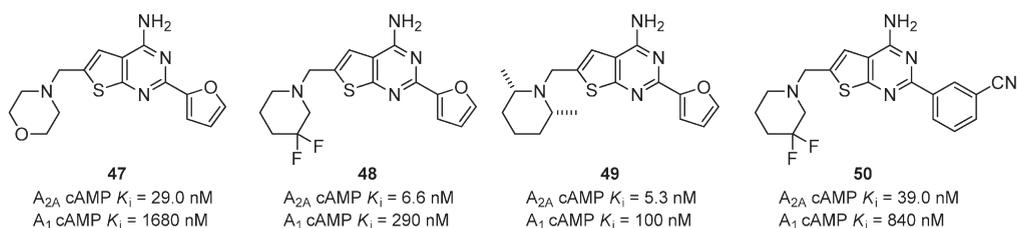


Figure 14. Aminomethyl substituted thieno[2,3-d]pyrimidines.

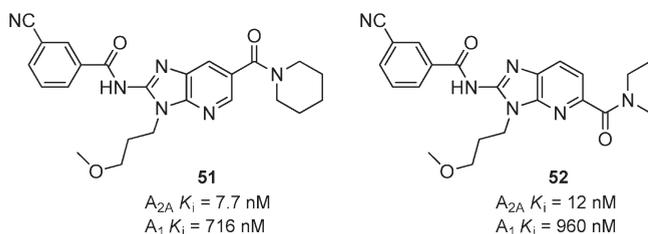


Figure 15. 2-Aminoimidazopyridines.

potent in vitro than 47 and significantly reversed catalepsy at 10 mg/kg, p.o. Further characterization of 48 showed that it had an ED₅₀ of <1 mg/kg, p.o., but suffered from a suboptimal PK profile that was attributed to the furan. Efforts to substitute the 5-position of the furan with methyl, ethyl, isopropyl, cyclopropyl, -CHF₂, chlorine, and bromine generally resulted in slight decreases of in vitro potency, but dramatic decreases of in vivo activity. Replacing the furan with various 5-membered heterocycles resulted in a very large decrease of in vitro potency. Interestingly, the furan could be replaced with a 3-cyanophenyl group and maintain good A_{2A} activity. Compound 50 was ~6-fold less potent than the corresponding furan analogue, but maintained good activity in vivo (ED₅₀ < 1 mg/kg) and had a superior PK profile.

A series of 2-aminoimidazopyridines 51 and 52 resulted from the optimization of a high throughput screen hit (Figure 15).⁵⁶ Compound 51 showed good binding affinity for A_{2A} with 93-fold selectivity over A₁. A variety of amides were explored in the 6-position but generally suffered from poor microsomal stability. Moving the amide substituent to the 5-position resulted in compounds with similar binding affinities, but in general led to better microsomal stability. Compound 52 was a potent binder for A_{2A} and showed moderate plasma and brain exposure when dosed orally, but it was not evaluated in animal models of PD.

A 4-morpholino-benzothiazole based series of compounds has been reported in the patent literature by Hoffmann-La Roche (Figure 16).⁵⁷ The imidazole 53⁵⁸ and the cyclohexane 54⁵⁹ are representative examples that each show good binding affinities for A_{2A}, but the A₁ activity was not reported. Further optimization of this scaffold led to the urea compound 55 which had good potency for A_{2A} and was 270-fold and >4000-fold selective versus A₁ in binding and functional assays, respectively. Compound 55 had an ED₅₀ of 0.5 mg/kg when dosed orally in the APEC-induced hypolocomotion model in rats.^{53,60} In January 2007, Synosia acquired the rights from Roche to develop 55 (SYN-115) for the treatment of PD.⁶¹ SYN-115 successfully completed a phase II clinical trial and is currently recruiting for additional phase II/III clinical trials for PD (clinicaltrials.gov identifier: NCT01283594) and for a phase 0 clinical trial for cocaine

dependence (clinicaltrials.gov identifier: NCT00783276). In February 2011, Synosia was acquired by Biotie Therapeutics.

A series of 4-aryl substituted benzofurans was identified, and the 4-phenyl-substituent was found to be the most consistent group for A_{2A} activity (Figure 17).⁶² The morpholine amide 56 showed good affinity for A_{2A} and significantly reversed (78%) CGS-induced catalepsy in mice at 10 mg/kg, p.o. Further exploration of this scaffold showed that the methyl carbamate 57 also had good A_{2A} activity and reversed catalepsy by 86% at 10 mg/kg, p.o. A variety of amides were prepared from a related 4-morpholinobenzofuran series and were evaluated in vitro and in vivo.⁶³ The analogues in general exhibited similar in vitro potencies with respect to the 4-phenylbenzofurans, but they also had increased aqueous solubility and improved PK properties. Compound 58 was identified having good in vitro activity for A_{2A} and excellent activity reversing (76% at 10 mg/kg, p.o.) CGS-induced catalepsy in mice. The compound also showed significant reduction in motor disabilities and increased locomotor activity in MPTP-treated marmosets for 5 h after a oral 10 mg/kg dose.

MONOCYCLIC A_{2A} ANTAGONISTS

An aminopyrazine based series of compounds was reported as dual A_{2A}/A₁ receptor antagonists (Figure 18).⁶⁴ Compound 59 had potent affinity for both A_{2A} and A₁ and significantly reversed haloperidol-induced catalepsy in mice at 3.2 mg/kg, p.o. Further optimization of the pyrazine scaffold led to the identification of 60 (ASP-5854) that has been characterized extensively in numerous animal models of PD and cognition.⁶⁵ ASP-5854 reversed CGS21680-induced and haloperidol-induced catalepsy in mice with ED₅₀ values of 0.15 and 0.07 mg/kg, p.o., respectively, and had a minimum effective dose of 0.1 mg/kg to reverse haloperidol-induced catalepsy in rats. In rhesus monkeys, ASP-5854 gave >85% occupancy of A_{2A} receptors in the striatum and reversed haloperidol-induced catalepsy with an ED₅₀ of 0.1 mg/kg, p.o.⁶⁶ ASP-5854 effectively potentiated L-DOPA-induced rotation in 6-OHDA lesioned rats with a minimum effective dose of 0.03 mg/kg, p.o. Statistical significance was reached in MPTP-treated mice and MPTP-treated marmoset models at 0.1 and 1 mg/kg, p.o., respectively.^{61,67} ASP-5854 produced positive results in scopolamine-induced memory deficits in the mouse Y-maze test and rat passive avoidance test, both models of cognition, with minimum effective doses of 0.3 and 0.1 mg/kg, p.o., respectively.⁶¹ The selective A_{2A} antagonist KW-6002 (3) was also tested in parallel for comparison in these models and was significantly less effective having a minimum effective dose of 10 mg/kg, p.o. in the mouse Y-maze test and no effect in the rat passive avoidance test up to 10 mg/kg, p.o. These results suggest that a dual A_{2A}/A₁ may provide added benefit to PD patients to address the cognitive impairments associated with the disease.

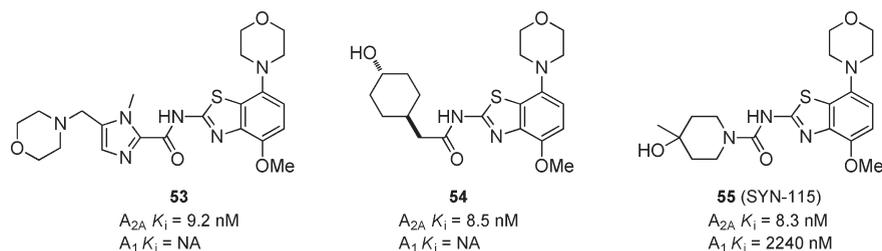


Figure 16. Substituted 4-morpholino-benzothiazoles.

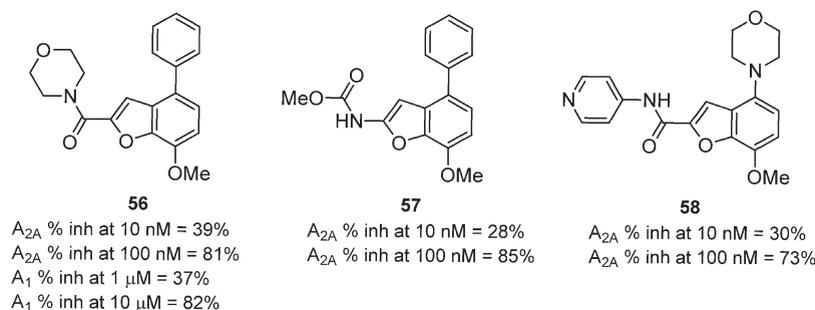


Figure 17. 4-Aryl and 4-morpholino substituted benzofurans.

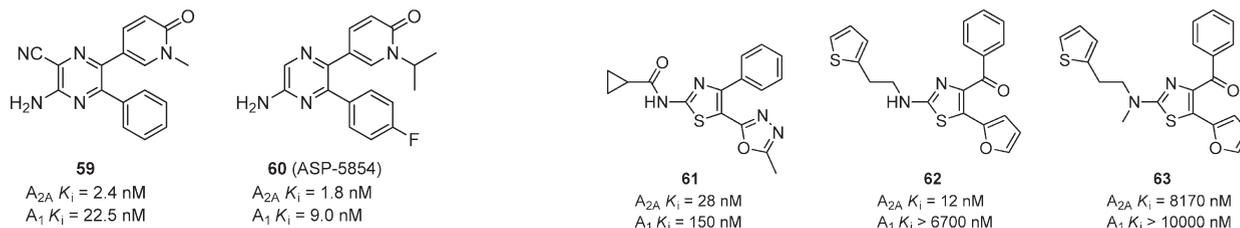


Figure 18. Pyridone substituted pyrazines.

Figure 19. Heterocyclic substituted 2-amino-thiazoles.

A variety of carboxamides of 2-amino-4-phenyl-thiazoles were prepared as adenosine A_{2A} receptor antagonists (Figure 19).⁶⁸ A lead optimization effort identified that the cyclopropyl amide was the optimal amide substituent. Further evaluation into the substitution at the 5-position of the thiazole led to the 5-methyl-[1,2,4]-oxadiazole compound **61** which had good affinity for A_{2A} and A_1 . Compound **61** also showed good activity in the haloperidol-induced hypolocomotion model in mice with an ED_{50} of 7 mg/kg. A series of related substituted 2-amino-5-benzoyl-4-(2-furyl)thiazoles was designed from a high throughput screening hit.⁶⁹ Lead optimization led to compound **62** which had good affinity for A_{2A} and excellent selectivity versus A_1 . Interestingly, a simple methyl substitution (**63**) dramatically decreases the binding affinity for A_{2A} . No in vivo evaluation of **62** was reported.

A series of trisubstituted pyrimidines was reported to have potent A_{2A} activity (Figure 20).⁷⁰ Compound **64** was a potent A_{2A} antagonist, but it lacked the desired selectivity versus A_1 and had poor metabolic stability that was attributed to the unsubstituted furan moiety. SAR studies resulted in the synthesis of the thiazole compounds **65** and **66** that maintained potency for A_{2A} . Interestingly, replacing the benzylic methylene, present in **65**, with an oxygen atom resulted in the selective compound **66**. Compound **66** was further optimized by replacing the thiazole with the dimethylpyrazole to give compound **67** that was very

potent for A_{2A} and had excellent selectivity versus A_1 . Despite the increase in selectivity, **67** suffered from poor solubility, so basic amines were appended to the phenol ring to increase solubility and improve PK properties. A number of the amino alkyl compounds had good binding affinities for A_{2A} and good selectivity versus A_1 , but had significant hERG liabilities. Compound **68** had the best hERG profile (patch clamp $IC_{50} = 1.2 \mu\text{M}$) and showed oral activity in the haloperidol-induced catalepsy in rats with a minimal effective dose of 10 mg/kg.

A series of 2-amino-*N*-pyrimidin-4-ylacetamides was prepared with compound **69** being the representative example (Figure 21).⁷¹ Compound **69** had a high affinity for A_{2A} and was 99-fold selective versus A_1 . Tissue distribution studies showed very good exposure (2700 ng/g) in rat brain at 2 h after a 10 mg/kg oral dose. The compound effectively reversed haloperidol-induced catalepsy in rats at 10 mg/kg, p.o. Like **64**, compound **69** had metabolic liabilities that were associated with the unsubstituted furan, so there was an effort to find a suitable furan replacement.⁷² A variety of combinations consisting of 5- and 6-membered heterocycles were made to replace the furan and pyrazole substituents. Compounds **70** and **71** were identified as the most promising compounds having good binding affinities and good selectivity versus A_1 . Further evaluation of **70** showed it to be a potent inhibitor of hERG with an IC_{50} of 950 nM (patch clamp). The basicity of the piperazine was enhancing the hERG

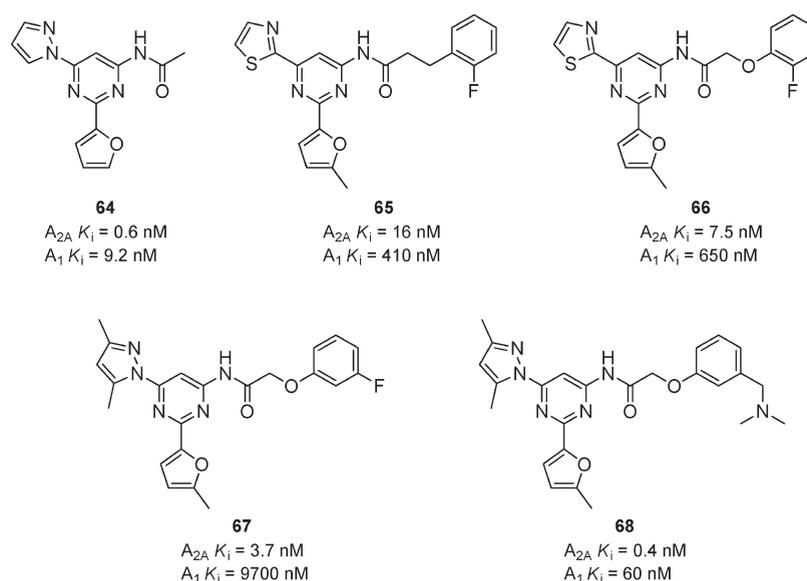


Figure 20. Various trisubstituted pyrimidines.

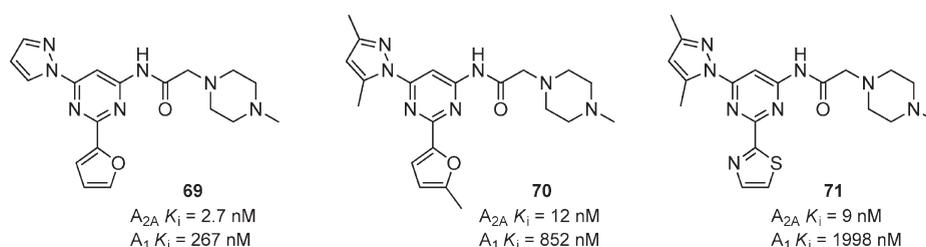


Figure 21. Piperazine substituted pyrimidine acetamides.

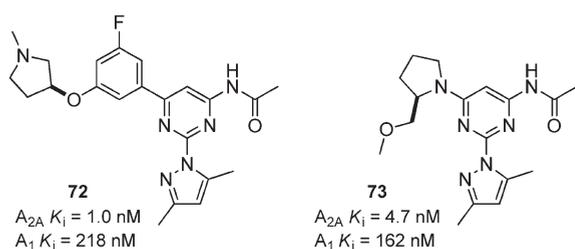


Figure 22. Acylaminopyrimidines.

activity, so various replacements were prepared in effort to decrease the hERG liability. Although the hERG inhibition could not be completely eliminated, removal of one of the basic amines decreased the hERG activity. Several compounds within the series showed activity in haloperidol-induced catalepsy in rats.

A related series of 2,6-diaryl-4-acylaminopyrimidines was developed with improved solubility and metabolic stability (Figure 22).⁷³ Compound **72** was potent for A_{2A} and was 218-fold selective against A_1 . More importantly, the addition of the fluorine atom significantly increased metabolic stability, decreased intrinsic clearance, and reduced CYP inhibition compared to the corresponding *des*-fluoro analogue (not shown). Further characterization of **72** showed that it significantly reversed haloperidol-induced catalepsy in rats at 30 mg/kg, p.o.

Another related compound **73** was able to reverse catalepsy in rats at 10 mg/kg, p.o. Compound **73** also showed the ability to potentiate L-DOPA-induced rotational behavior in the 6-OHDA lesioned rat at 10 and 30 mg/kg oral doses.

Analogues of pyrimidine-4-carboxamides have been reported to display good potency for A_{2A} and with good selectivity over other adenosine receptor subtypes (Figure 23).⁷⁴ Compound **74** is a representative example having binding affinities of 1.7, 43, 460, and 1740 nM for A_{2A} , A_1 , A_{2B} , and A_3 , respectively. Compound **75** also had a minimum effective dose of 0.1 mg/kg in the haloperidol-induced hypolocomotion model in mice. Related pyridine **75** and triazine **76** have also been reported, but they are significantly less potent than the corresponding pyrimidines.⁷⁵

SUMMARY

Adenosine A_{2A} receptor antagonists continue to be a sought after target for the treatment of PD. There have been a variety of chemical scaffolds reported with interesting SAR features that showed dramatic activity differences, both in vitro and in vivo, from subtle modifications. Despite the relentless research efforts to identify potent compounds, there still remains the challenge of achieving selectivity, solubility, and acceptable pharmacokinetic/pharmacodynamic properties to progress into the clinic. There have been a number of compounds that are in or have been in clinical trials including istradefylline (**3**), preladenant (**8**),

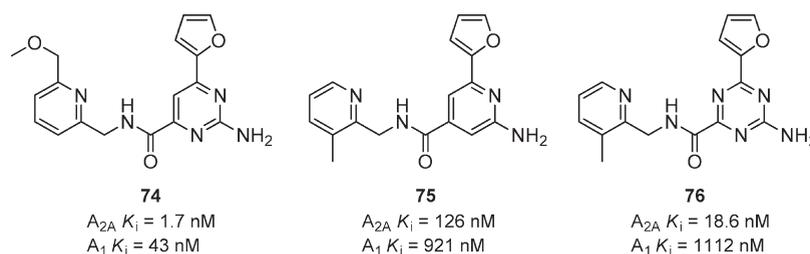


Figure 23. Pyrimidine, pyridine, and triazine carboxamides.

vipadenant (**44**), ST-1535 (**46**), and SYN-115 (**55**). The progression through the clinic with positive trial results indicates the viability of A_{2A} antagonists to treat PD patients with potential advantages over the current standard treatments. Two examples of efforts to identify dual A_{2A}/A_1 were also described as having the potential to provide added benefit to PD patients, via A_1 antagonism, to address the cognitive impairments that worsen as the disease progresses. Indenopyrimidine **18** and ASP-5854 (**60**) are potent A_{2A}/A_1 receptor antagonists that showed very potent activity across a number of PD models. ASP-5854 also showed potent and effective activity in a number of rodent cognition models, while the selective A_{2A} antagonist istradefylline showed no or marginal effects in these models. The continued research to identify selective A_{2A} and dual A_{2A}/A_1 antagonists should provide some new and interesting opportunities for PD patients for years to come.

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ABBREVIATIONS

6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; DA, dopamine; L-DOPA, levodopamine; MAO-B, monoamine oxidase type B; COMT, catechol *O*-methyl-transferase; cAMP, cyclic adenosine monophosphate; PK, pharmacokinetics

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The table of contents graphic represents a positron emission tomography (PET) picture of adenosine A_{2A} receptors in rat striatum using ^{11}C -TMSX, an A_{2A} receptor antagonist. We thank Dr. Paul Acton and Carlos Cotto for performing the PET experiments.

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